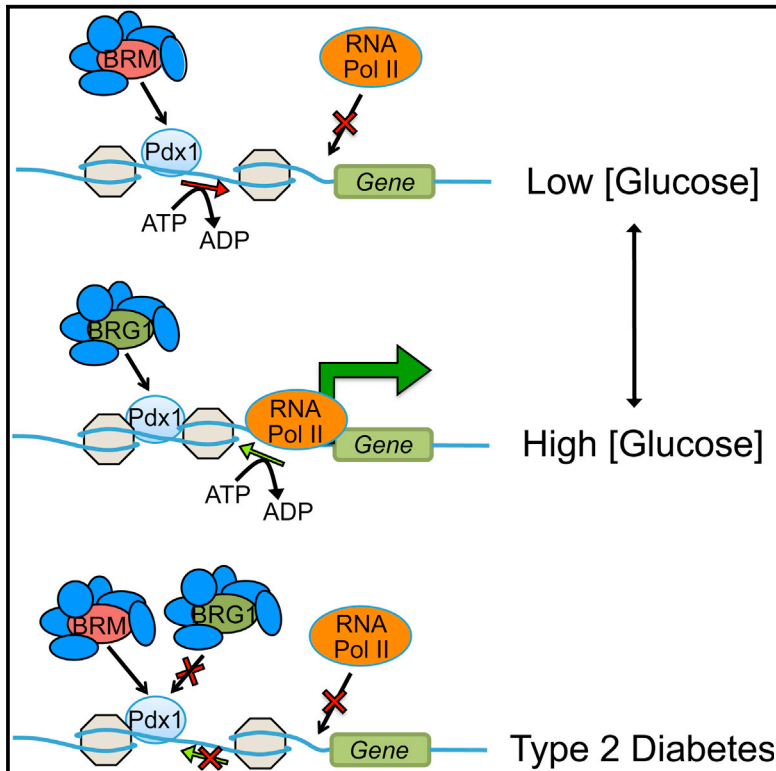


# Cell Reports

## Dynamic Recruitment of Functionally Distinct Swi/Snf Chromatin Remodeling Complexes Modulates Pdx1 Activity in Islet $\beta$ Cells

### Graphical Abstract



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### In Brief

PDX1 is a transcription factor of fundamental importance to pancreas development and  $\beta$  cell function. Here, McKenna et al. show how context-dependent recruitment of coregulatory complexes impacts PDX1 activity, thus linking ATP-dependent chromatin remodeling to control of islet  $\beta$  cell function under normal and disease conditions.

### Highlights

- Numerous Pdx1 coregulators with unique cellular functions are identified
- Pdx1 dynamically interacts with distinct Swi/Snf complexes in islet  $\beta$  cells
- Physiological and pathophysiological conditions influence Swi/Snf recruitment



# Dynamic Recruitment of Functionally Distinct Swi/Snf Chromatin Remodeling Complexes Modulates Pdx1 Activity in Islet $\beta$ Cells

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## SUMMARY

Pdx1 is a transcription factor of fundamental importance to pancreas formation and adult islet  $\beta$  cell function. However, little is known about the positive- and negative-acting coregulators recruited to mediate transcriptional control. Here, we isolated numerous Pdx1-interacting factors possessing a wide range of cellular functions linked with this protein, including, but not limited to, coregulators associated with transcriptional activation and repression, DNA damage response, and DNA replication. Because chromatin remodeling activities are essential to developmental lineage decisions and adult cell function, our analysis focused on investigating the influence of the Swi/Snf chromatin remodeler on Pdx1 action. The two mutually exclusive and indispensable Swi/Snf core ATPase subunits, Brg1 and Brm, distinctly affected target gene expression in  $\beta$  cells. Furthermore, physiological and pathophysiological conditions dynamically regulated Pdx1 binding to these Swi/Snf complexes *in vivo*. We discuss how context-dependent recruitment of coregulatory complexes by Pdx1 could impact pancreas cell development and adult islet  $\beta$  cell activity.

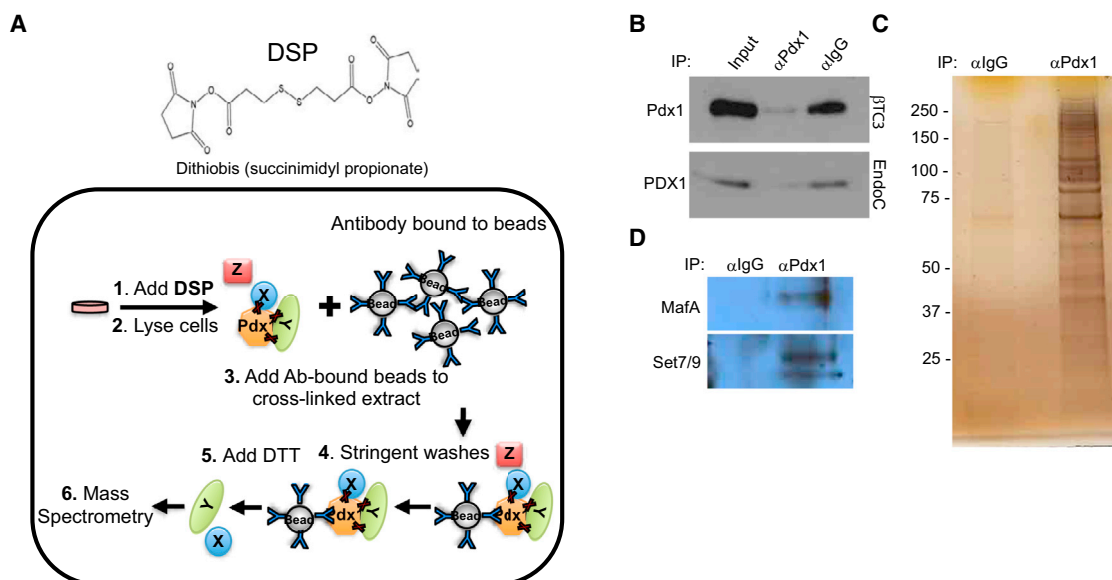
## INTRODUCTION

The Pdx1 protein has profound transcriptional regulatory properties and is an essential driver of normal pancreas development and mature  $\beta$  cell function. For example, embryonic formation of ductal, acinar, and islet cells that compose the pancreas are derived from a common Pdx1-expressing progenitor pool (Jonsson et al., 1994; Offield et al., 1996), with pancreas agenesis manifested in mice and humans unable to produce the functional protein (Jonsson et al., 1994; Offield et al., 1996; Stoffers et al., 1997). Developing mouse  $\beta$  (Gannon et al., 2008) and/or acinar cell (Hale et al., 2005) formation is also severely blunted upon

Pdx1 removal from the progenitors of these cell populations. Moreover, forced and persistent Pdx1 expression in embryonic Neurogenin 3 (Ngn3)<sup>+</sup> pan-endocrine cell progenitors causes islet glucagon hormone<sup>+</sup>  $\alpha$  cells to become insulin<sup>+</sup>  $\beta$  cells postnatally (Yang et al., 2011). Conversely, specific deletion of Pdx1 from adult islet  $\beta$  cells, which are the principal adult pancreatic cell type producing this protein, results in their rapid acquisition of an  $\alpha$ -like cell identity (Gao et al., 2014).

Like all transcription factors, Pdx1 binds to specific *cis*-acting sequences within target gene control regions, often referred to as enhancer regions (Pasquali et al., 2014). Transcriptional modulation is then imposed through dynamic changes in chromatin structure that causes the underlying DNA to become more or less accessible to RNA polymerase II. However, even powerful transcription factors like Pdx1 lack inherent chromatin-modifying properties and alone cannot modulate gene transcription effectively. Instead, cell-signaling events drive context-dependent protein-protein interactions between these DNA-binding factors and coregulators, which possess transcriptional modifying functions. For example, acute exposure of  $\beta$  cell lines to glucose, the most important physiological effector of cell function *in vivo*, causes a rapid activator/repressor switch in Pdx1 activity on *Insulin* gene transcription. This involves the differential recruitment of histone acetyltransferases (HAT) coactivator at high, stimulating glucose concentrations, and histone deacetylase (HDAC) corepressors at low, inhibitory glucose levels (Mosley and Ozcan, 2004; Mosley et al., 2004).

Coregulators that alter chromatin structure can do so by both enzymatic and non-enzymatic means. Non-enzymatic coregulators such as Mediator harbor protein-protein and protein-DNA/RNA interaction surfaces that influence transcription by altering epigenetic patterns, chromatin compaction, as well as the recruitment of distinct cofactors and RNA Polymerase II (Poss et al., 2013). The coregulators that function enzymatically can be divided into two main mechanistically distinct classes. First are those that alter chromatin through covalent modifications to DNA (e.g., methylation) and DNA-binding proteins (e.g., histones, transcription factors, and coregulators by acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and/or glycosylation) (Bhaumik et al., 2007; Chen and Li, 2004; Flotho and Melchior, 2013; Wells et al., 2003). Second are those that use the energy of ATP hydrolysis to destabilize nucleosomes



**Figure 1. ReCLIP Enriches for Pdx1-Interacting Proteins**

(A) Diagram of ReCLIP showing the lysine-reactive cross-linking reagent, DSP, that is first incubated with  $\beta$  cells from which nuclear extract is generated for Pdx1 antibody or IgG immunoprecipitation. The control and  $\alpha$ -Pdx1-bound complexes were washed with RIPA buffer and then RIPA + DTT to elute bound cross-linked proteins.

(B) Nearly all of Pdx1 was removed from the coIP supernatants with Pdx1 antibody.

(C and D) The eluted proteins were (C) SDS-PAGE separated and submitted for MS analysis or (D) immunoblotted with MafA or Set7/9 antibodies (Tables 1 and S1) ( $n \geq 3$ ).

and alter the accessibility of DNA to the transcriptional machinery (Sudarsanam and Winston, 2000). While there are over 250 transcriptional coregulators in mammalian cells, relatively few have been ascribed to Pdx1 specifically (Pcif1, p300, HDAC1/2, Set7/9, and Bridge1 [Francis et al., 2005; Liu et al., 2004; Mosley and Ozcan, 2004; Qiu et al., 2002; Stanojevic et al., 2005]) or other islet-enriched transcription factors like Nkx2.2 (Grg3, HDAC1, and DNMT3a [Papizan et al., 2011]), Isl1 (Ldb1/2 [Hunter et al., 2013]), HNF1 $\beta$  (PCAF/CBP [Barbacci et al., 2004]), and NeuroD1 (Bridge1 and p300/CBP [Qiu et al., 1998; Thomas et al., 1999]). Significantly, essentially all of these transcription factor associations were made in studies using a small subset of candidate coregulators.

Here we have used an unbiased chemical cross-linking, antibody precipitation, and mass spectrometry (MS) strategy to identify endogenous Pdx1-binding proteins in  $\beta$  cells. Although many new and interesting coregulatory factors were found using this in-cell cross-linking approach, we chose to specifically focus on investigating whether Swi/Snf chromatin remodeling complex recruitment was linked to the positive- and/or negative-acting control properties of Pdx1. Our results strongly suggest that Pdx1 interacts with functionally distinct Swi/Snf complexes in a highly dynamic manner in islet  $\beta$  cells. Hence, Swi/Snf complexes containing the core Brg1 ATPase subunit were demonstrated to be involved in Pdx1-mediated activation, while the Brm ATPase subunit containing complexes imposed transcriptional repression. Evidence is also presented indicating that physiological and pathophysiological conditions influence Pdx1 binding to these distinct complexes in  $\beta$  cells in vivo, and

we propose that the antagonistic actions of Brg1-Swi/Snf and Brm-Swi/Snf have significant implications to glucose homeostasis. Our findings have identified many different possible coregulators of Pdx1 and shed light on how their recruitment and consequent transcription factor function is impacted normally and in the context of dysfunctional type 2 diabetes mellitus (T2DM)  $\beta$  cells.

## RESULTS

### Pdx1 Interacts with Many Functionally Distinct Coregulatory Proteins in $\beta$ Cells

An unbiased strategy of reversible cross-linking, co-immunoprecipitation (ReCLIP [Smith et al., 2011]) and MS was used to identify Pdx1-interacting proteins in  $\beta$ TC3 cells, a murine  $\beta$  cell line (Figure 1A). Employment of the cell-permeable, lysine-reactive, thiol-cleavable dithiobis(succinimidyl propionate) (DSP) cross-linking reagent allowed for stringent detergent-based washes, which led to a very clear difference in the proteins obtained from the Pdx1 antibody and control precipitates (Figures 1B and 1C). As anticipated, established Pdx1-interacting factors such as the MafA transcription factor (Zhao et al., 2005) and the Set7/9 coregulator (Francis et al., 2005) were found by immunoblot analysis after Pdx1 precipitation (Figure 1D). Additionally, we identified a number of novel candidate Pdx1-binding factors by MS (Table 1; Table S1). Many of the candidates are likely linked with Pdx1 transcriptional regulation, while others may be associated with context-dependent recruitment in, for example,

**Table 1. Pdx1-Bound Chromatin Remodeling Complexes Identified by ReCLIP/MS**

Coregulator	Protein	Coverage (%)	Peptides	
			IgG Control	Pdx1 IP
NuRD complex	Rbbp4	9	1	10
	Hdac1/2	4	0	5
	Mi-2 $\beta$	3	0	7
Swi/Snf complex	Baf47	11	0	1
	Baf155	3	0	3
	Baf57	9	0	2
	Baf170	3	0	3
	Brg1	2	0	5
Tif1 $\beta$	Tif1 $\beta$	14	0	6

The coregulator complex is shown as well as the MS results illustrating percentage protein subunit coverage and the number of peptides found by IgG or Pdx1 antibody precipitation. These results are representative of data averaged from at least three independently performed IgG and Pdx1 antibody treatments.

DNA repair, proliferation, and apoptosis (Babu et al., 2007; Leb-run et al., 2005).

Several of the transcriptional coregulators were of particular interest because of their ability to potentially activate and/or repress Pdx1 *trans*-regulation (e.g., Tif1 $\beta$ , Swi/Snf, and NuRD) (Flowers et al., 2009; Iyengar et al., 2011; Miccio et al., 2010). The binding of Tif1 $\beta$ , Swi/Snf, and NuRD complex proteins to Pdx1 was independently confirmed in co-immunoprecipitation (coIP) experiments performed with both mouse and human  $\beta$  cell lines (Figure 2A). The authenticity of the interactions was further verified by the ability to precipitate Pdx1 with specific coregulator antibodies (Figure 2B). In addition, the roughly 42 kDa Pdx1 protein was found to migrate as a high molecular weight complex after sucrose gradient sedimentation of  $\beta$ TC3 nuclear extracts (Figure 2C), and to bind to Swi/Snf complex subunits by coIP analysis (Figure 2E). Moreover, Pdx1-Swi/Snf was shown to retain specific *cis*-control-element-binding properties in electrophoretic mobility shift assays (EMSAs) (Figure 2D). Notably, few of the published Pdx1 coregulators and none of the interacting islet-enriched transcription factors were detected by MS (Table 1; Table S1). We presume that these represent relatively rare complexes observed by more sensitive analytical methods (e.g., immune-based analysis). Significantly, these experiments identified a number of new, interesting interacting candidate proteins involved in Pdx1 action.

### Pdx1 Interacts in the Developing and Adult Pancreas with Brg1 and Brm, the Catalytic ATPase Subunits of the Swi/Snf Complex

The contribution of Swi/Snf to Pdx1 activity was further investigated because of the prominent actions of this coregulator in developing neuron, cardiomyocyte, and lymphocyte cell proliferation and lineage decisions (Chi et al., 2003; Lessard et al., 2007; Lickert et al., 2004). Immunofluorescence analysis revealed that the ATPase subunits of Swi/Snf, Brg1 and Brm, were broadly expressed in the embryonic and adult pancreas, with no observable difference in protein level between cell types within each

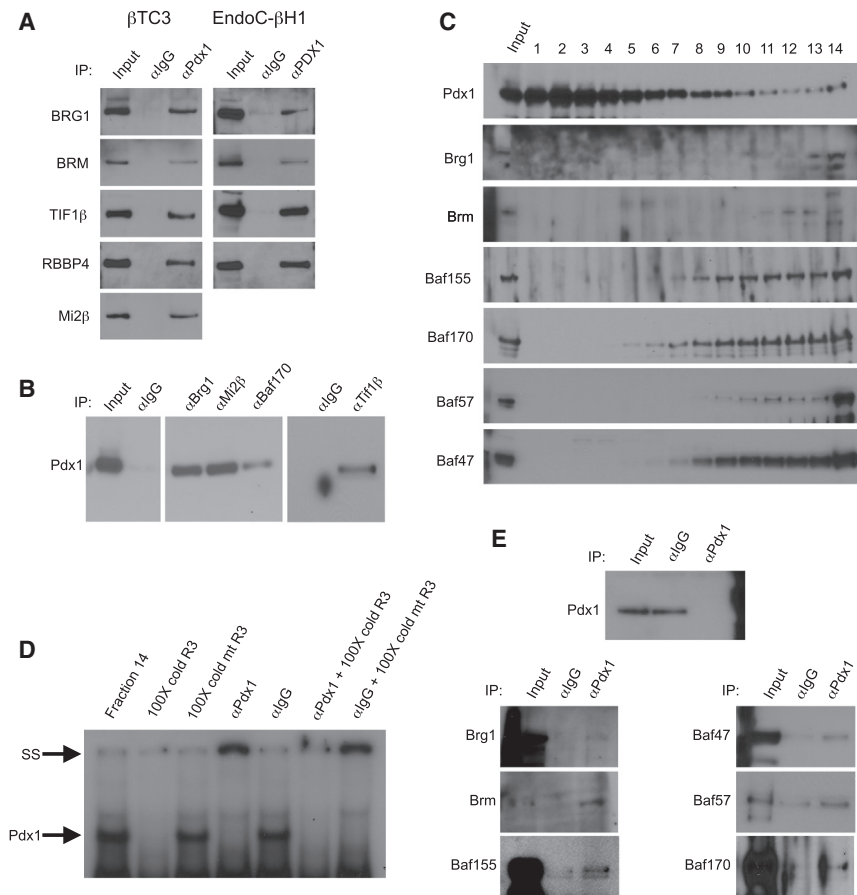
developmental stage (Figures S2A and S2B). Because of the unique and dynamic expression pattern of Pdx1 in the pancreas, this transcription factor would be coexpressed with Brm- and Brg1-Swi/Snf within multipotent pancreatic progenitor cells during early embryogenesis (i.e., embryonic day E12.5 [Offield et al., 1996]), and then principally in the insulin<sup>+</sup> cells of developing (E15.5, E18.5) and adult islet  $\beta$  cells that express relatively high levels of Pdx1 (termed Pdx1<sup>High</sup> [Boyer et al., 2006]), and not acinar, ductal, or other islet cell types (i.e.,  $\alpha$ ,  $\epsilon$ ,  $\delta$ , and PP) (Guz et al., 1995; Ohlsson et al., 1993).

The proximity ligation assay (PLA) was next used to evaluate Pdx1 binding to Brg1 and Brm in the forming and adult pancreas. In this assay, a fluorescent signal is generated if Pdx1 is within 30–40 nm of these Swi/Snf proteins, a physical distance only producing a signal for neighboring proteins. However, the signals are not a reflection of the absolute quantity of Pdx1:Brg1-Swi/Snf or Pdx1:Brm1-Swi/Snf complexes in a cell due to antibody quality and/or other vagaries of the assay. Significantly, Pdx1:Brg1 and Pdx1:Brm signals were detected in nearly all Pdx1<sup>+</sup> pancreatic progenitors of the E12.5 pancreatic epithelium (Figures S3A and S3B). In contrast, no Brg1 or Brm PLA binding was observed using antibodies specific for Sox9 (Seymour et al., 2007) or Ptf1a (Krapp et al., 1998), other key transcription factors enriched in this multipotent progenitor population (data not shown). Notably, Pdx1:Brg1 and Pdx1:Brm signals became confined to insulin<sup>+</sup> cells later in development and in islets (Figures S3A and S3B). These experimental results demonstrate that Pdx1 interacts with the core catalytic subunits of Swi/Snf in the developing and adult mouse pancreas, which we conclude represents greater Swi/Snf complex recruitment. Furthermore, the progressive restriction of binding to insulin<sup>+</sup> cells supports the specific nature of these interactions, and implies functional relevance upon consideration of the many established roles of Swi/Snf in other cell types (Chi et al., 2003; Lessard et al., 2007; Lickert et al., 2004).

### Blood Glucose Concentration Dynamically Regulates Pdx1:Brg1 Levels in Mouse Islet $\beta$ Cells

We next asked whether Pdx1:Swi/Snf complex formation was regulated in a glucose-dependent manner *in vivo* by comparing PLA signal numbers in pancreata prepared from fasted mice with low blood glucose levels to those fasted then given an intraperitoneal injection of a high-glucose solution (Figure 3A). Strikingly, the number of Pdx1:Brg1 complexes was significantly increased compared to fasted and fed (ad libitum) controls 30 min after glucose treatment, as shown quantitatively by the specific increase per  $\beta$  cell nucleus after high-glucose treatment (Figures 3B and 3C). Additionally, fed (ad libitum) and fasted mouse islets had roughly 3- and 5-fold more  $\beta$  cells displaying zero detectable PLA signals than glucose-injected animals (Figure 3D). These data illustrate a strong, positive relationship between high-glucose conditions that stimulate Pdx1  $\beta$  cell activity and Pdx1:Brg1 binding (Figure 3C). Supporting the specific nature of these interactions, no PLA signals were detected under these conditions between Pdx1 and Isl1 transcription factor coregulator, Ldb1 (Hunter et al., 2013; Figure S4). Interestingly, although the Pdx1:Brm signals changed some under these conditions, relatively few of the data points were significantly different





**Figure 2. Proteins in NuRD, Tif1β, and Swi/Snf Immunoprecipitated with Pdx1 in Mouse βTC3 and Human EndoC-βH1 Cells**

(A) Proteins of the Swi/Snf (Brg1 and Brm) and NuRD (Rbbp4 and Mi2) complexes and Tif1β were enriched in Pdx1 antibody ReCLIP precipitations of βTC3 (left) and EndoC-βH1 (right) extracts. (B) Reverse colPs illustrate Pdx1 enrichment in βTC3 nuclear extracts immunoprecipitated with Tif1β, Swi/Snf, and NuRD coregulator subunit antibodies (Figure S1). (C–E) Sucrose gradient sedimentation demonstrates that the Pdx1 (C) migrating in the highest molecular weight fraction retains the ability to (D) bind Pdx1-regulated control element DNA and (E) coprecipitate with SWI/SNF complex subunits ( $n \geq 3$  for each colP).

BRM-SWI/SNF represses transcription of differentiation genes such as *osteocalcin* in osteoblast progenitors, at least in part through recruitment of the HDAC1 corepressor. In contrast, BRM and HDAC1 dissociate from the *osteocalcin* promoter during differentiation, allowing activating BRG1-specific complexes to initiate transcription. These experiments reveal how recruitment of BRG1- or BRM-containing SWI/SNF complexes can produce different transcriptional outcomes.

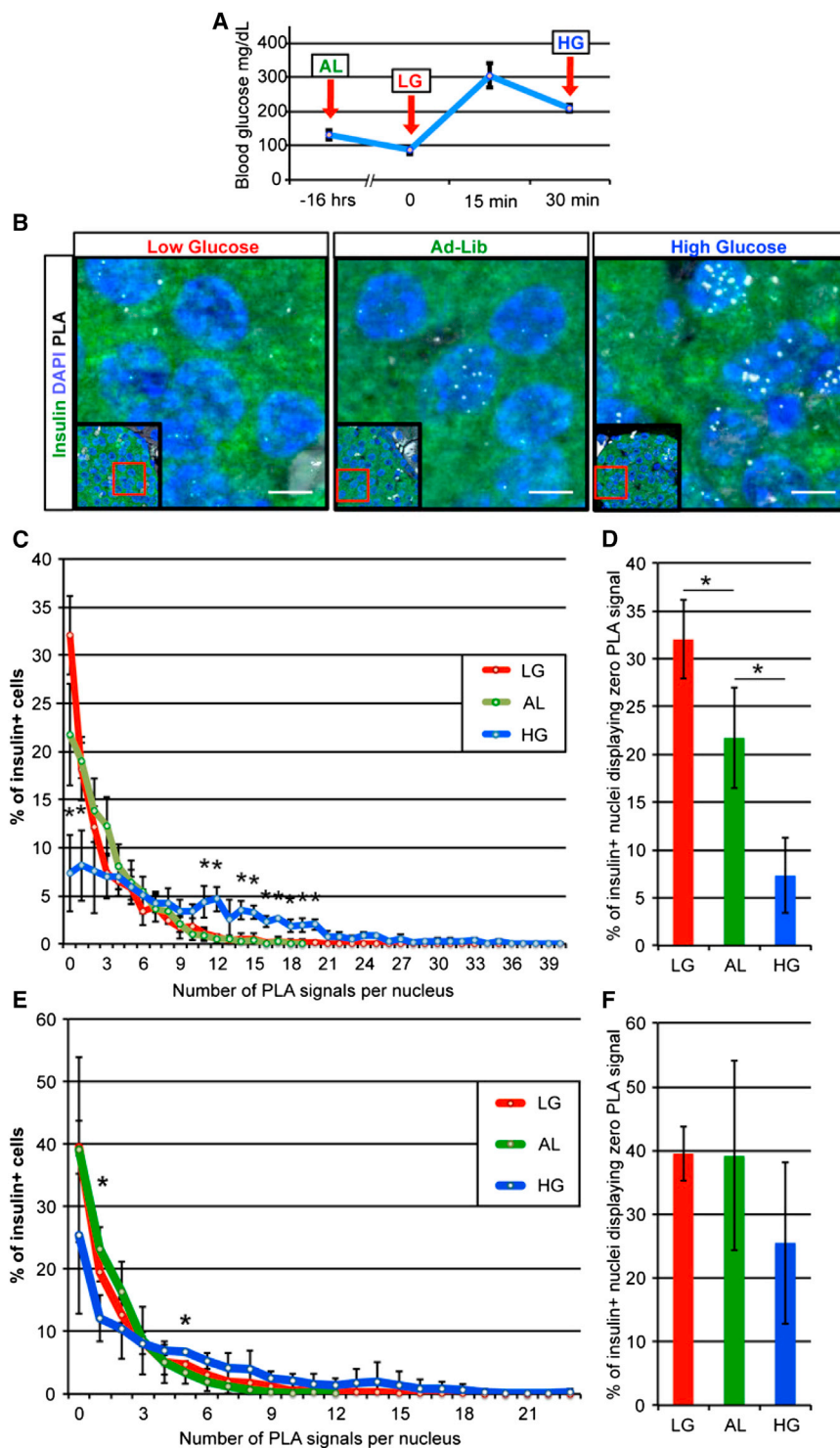
Pdx1 both activates and represses gene transcription in islet β cells (Gao

et al., 2014). Quantitative chromatin immunoprecipitation (ChIP)-qPCR assays were performed in mouse Min6 β cells with Brg1- and Brm-specific antibodies to examine their pattern of recruitment to genes that Pdx1 either directly stimulates (i.e., β cell-enriched *Insulin*, *MafA*, and *Pdx1*) or inhibits (i.e., islet α cell-enriched *MafB* and *Glucagon* [Artnr et al., 2006; Gao et al., 2014]). Brg1 was recruited to genes stimulated by Pdx1 in β cells (Figure 4). However, antibody reagent limitations precluded our ability to detect Brm control region binding, as illustrated by our inability to even detect control, bona fide Brm-binding sites in this assay (Figure S5A).

### Brg1 and Brm Play Opposing Roles in the Regulation of β-Cell-Specific Genes

Brg1 or Brm are incorporated into Swi/Snf complexes in a mutually exclusive manner (Wang et al., 1996). These two ATPases share ~74% amino acid sequence identity and have similar in vitro biochemical activity (Khavari et al., 1993; Phelan et al., 1999). Brg1 and Brm have the capacity to compensate for each other in heterozygous ATPase subunit mutant mice during development, although their combined gene dosage is critical to function (Bultman et al., 2000; Smith-Roe and Bultman, 2013; Willis et al., 2012). Null mutants of nearly ubiquitously expressed Brg1 and Brm manifest different phenotypes in vivo. Thus, *Brg1*<sup>−/−</sup> mice die at the preimplantation stage of development (Bultman et al., 2000), while *Brm*<sup>−/−</sup> mice survive to adulthood with only a roughly 15% increase in body mass, which is attributed to greater bone density (Reyes et al., 1998). Intriguingly, these ATPases have distinct and antagonistic roles in human osteoblast formation, with depletion of BRG1 hindering differentiation and BRM accelerating it (Flowers et al., 2009).

Depletion of Pdx1 from either islet β cells in vivo or β cell lines causes a rapid loss in cell identity marker expression (e.g., *MafA*, *Glut2*, and *insulin*), and activation of islet α cell-specific genes (e.g., *MafB* and *glucagon*) (Gao et al., 2014). The induction of islet α cell-enriched *MafB* transcription factor expression was a driving force in *glucagon* transcription. These studies clearly illustrate the critical role of Pdx1 in maintaining β cell identity. We therefore sought to investigate how depletion of Brg1 or Brm in rat INS-1 β cells influenced Pdx1-mediated transcriptional control. Brg1 and Brm small interfering RNA (siRNA) targeting decreased protein levels by roughly 65% (Figure 5A) and 55% (Figure 5C), respectively. As expected from the ChIP results, Brg1 knockdown resulted in a significant reduction in *Insulin*, *MafA*, and *Glut2* transcript levels (Figure 5B). Interestingly,



**Figure 3. Pdx1:Brg1 Complex Formation Is Acutely Regulated by Blood Glucose Concentration In Vivo**

(A) Diagram showing mouse blood glucose concentration and time of ad libitum (AL), low-glucose (16-hr fast [LG]), and high-glucose (16-hr fast + glucose injection [HG]) conditions; arrows represent time of sacrifice and pancreas fixation.

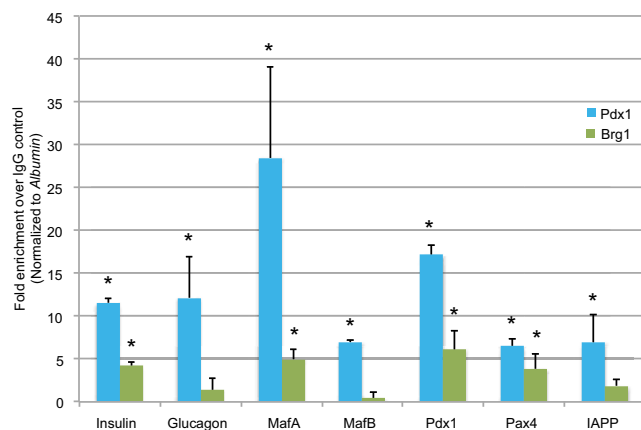
(B) Pdx1:Brg1 PLA/immunofluorescence analysis of islet  $\beta$  cell nuclei reveals that Pdx1:Brg1 signals become more abundant with increasing blood glucose concentration. Representative images are shown with PLA signals appearing as white spots; lower magnification insets are provided to orient each image. Scale bars, 5  $\mu$ M.

(C–F) (C) The distribution of insulin<sup>+</sup> PLA signals under high-glucose conditions were significantly greater than in low or ad libitum  $\beta$  cells, although the (E) Pdx1:Brm population was essentially unchanged. Islets from high-glucose conditions possessed significantly fewer  $\beta$  cells displaying zero detectable (D) Pdx1:Brg1 signals than low or ad libitum conditions. This pattern was not observed for (F) Pdx1:Brm between glucose conditions ( $n \geq 3$ , \* $p < 0.05$ ). Error bars represent SD.

### PDX1 Recruitment of BRG1-SWI/SNF Is Compromised in Human T2DM Islet $\beta$ Cells

Reduced levels of a small subset of islet-enriched transcription factors contribute to T2DM  $\beta$  cell dysfunction, with PDX1 among the specifically affected factors (Guo et al., 2013). We therefore sought to investigate whether alterations in PDX1:SWI/SNF complex formation could be contributing to diminished  $\beta$  cell activity. PDX1 binding to BRG1 (Figure 6A) and BRM (Figure 6D) was readily detected in normal human islet  $\beta$  cell nuclei. However, only insulin<sup>+</sup> PDX1:BRG1 (Figure 6B) signal numbers were significantly diminished in age-, sex-, and BMI-matched T2DM samples, and not PDX1:BRM (Figure 6E). The change in PDX1:BRG1 levels paralleled that of PDX1 in T2DM islet  $\beta$  cells (Guo et al., 2013), whereas there was no apparent change in BRG1 or BRM levels compared to normal tissue (Figures S2C and S2D). In addition, there was a roughly 2-fold increase in the number of  $\beta$  cells with zero PLA signals in the PDX1:BRG1 T2DM samples (Figure 6C),

even though PDX1:BRM levels were unaffected (Figure 6F). This suggests that the quantitative change in the amount of PDX1 protein affects BRG1-SWI/SNF coactivator binding in T2DM  $\beta$  cells, and not the BRM-SWI/SNF corepressor. Collectively, these results strongly indicate that the association of



**Figure 4. Brg1 Binds to Pdx1-Activated Genes in Min6  $\beta$  Cells**

ChIP assays illustrating Pdx1 and Brg1 genomic binding, with Brg1 enriched at activated (*Insulin*, *MafA* R3, *Pdx1* A111, and *Pax4*) Pdx1 target genes in Min6 cells. Notably, Brg1 did not bind to these control regions in Brg1<sup>+</sup>, PDX1<sup>−</sup> HeLa cells (Figure S5B) (n = 4, \*p < 0.05). Error bars represent SD.

PDX1 to the SWI/SNF chromatin remodeler is dynamically regulated under both physiological and pathophysiological conditions in islet  $\beta$  cells.

## DISCUSSION

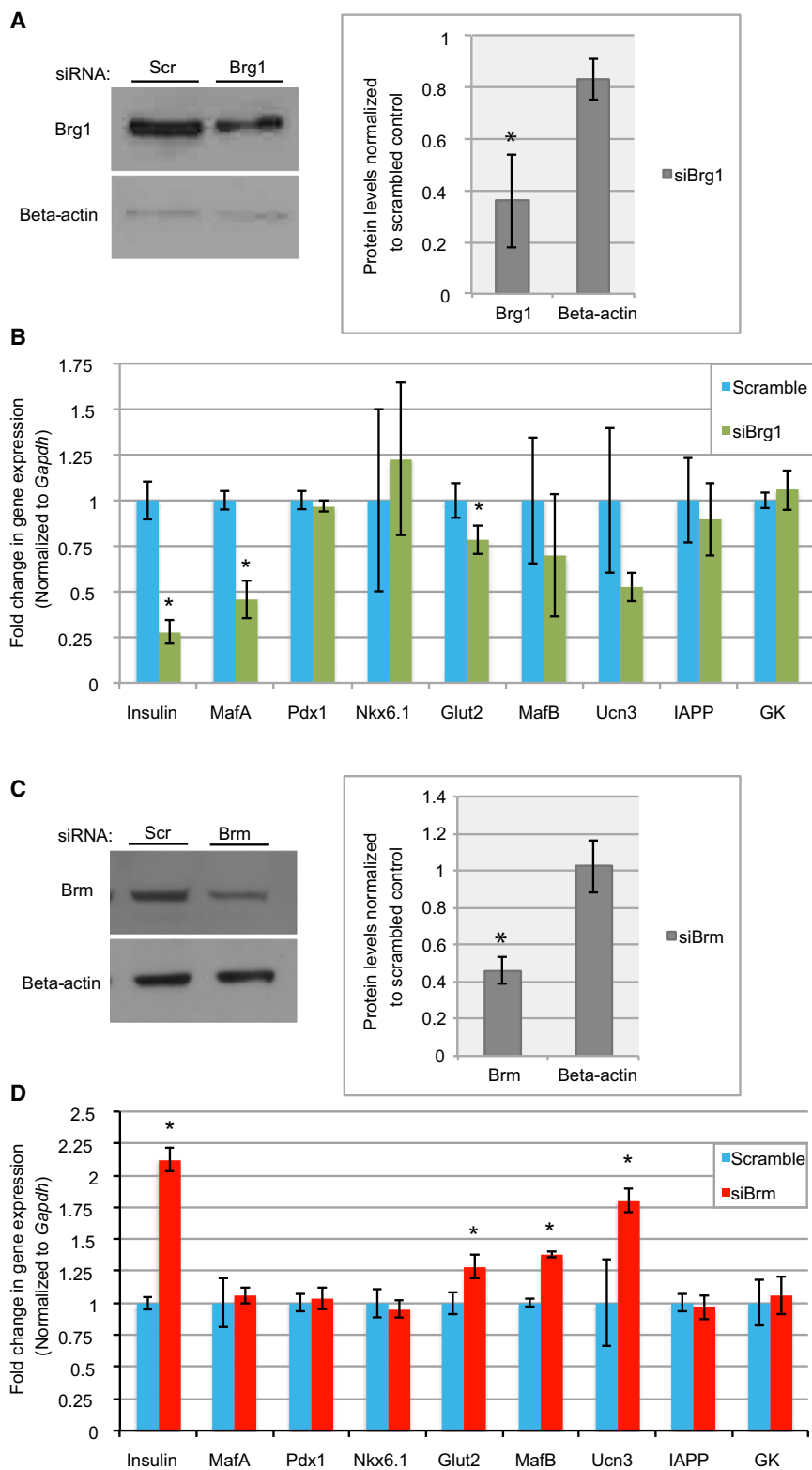
Modulation of target gene expression by transcription factors is contingent upon their ability to recruit coregulator proteins, such as those with a capacity to modify chromatin structure. Surprisingly, despite the fundamental importance of transcription factors such as Pdx1, MafB, Nkx2.2, and Nkx6.1 to islet  $\beta$  cell development and function, there is a glaring deficiency in our knowledge of how coregulators influence these and other islet-enriched transcription factors. Thus, the goal here was to generate an impartial and comprehensive list of coregulators binding to Pdx1 in  $\beta$  cells. Our ReCLIP/MS screen returned a substantial number of candidate coregulators with a vast array of cellular functions, including several capable of mediating the positive and negative transcriptional actions linked to Pdx1 (e.g., TIF1 $\beta$  [Iyengar et al., 2011], DNA methyltransferase 1 or Dnmt1 [Dhawan et al., 2011], NuRD [Miccio et al., 2010], and Swi/Snf [Flowers et al., 2009]) (Figure 2; Tables 1 and S1). We focused our analysis on determining the significance of the Pdx1:Swi/Snf complex in modulating  $\beta$  cell activity. Our results strongly suggest that recruitment of Swi/Snf complexes harboring the Brg1 ATPase is essential for Pdx1 transactivation, while the Brm-Swi/Snf complexes mediate repression (Figure S6).

Brg1 and Brm were widely produced throughout the pancreatic epithelium and in the surrounding mesenchyme of the E12.5 pancreas (Figures S2A and S2B). PLA analyses revealed Pdx1 interacts with both ATPases in nearly all cells of the multipotent Pdx1<sup>+</sup> epithelium but not in the surrounding mesenchyme where Pdx1 is not produced (Figures S3A and S3B). Notably, a hypoplastic pancreas phenotype was found in mice upon the removal of floxed *Brg1* early in pancreas development

by *Ptf1a-Cre* (von Figura et al., 2014). Interestingly, all of the mature pancreatic cell types were formed and *Brg1* mutant animals were otherwise healthy, albeit final pancreas size was reduced by 50%. Because the number of multipotent progenitor cells limits pancreas size (Stanger et al., 2007), we propose that reduced Brg1 decreases Pdx1<sup>+</sup>/Ptf1a<sup>+</sup>/Sox9<sup>+</sup> progenitor numbers by affecting their proliferative capacity and/or health. Moreover, we further suggest that this is principally due to the actions of Pdx1 recruitment of Swi/Snf to multipotent progenitor cell target genes, and not Ptf1a or Sox9, as neither associated with Swi/Snf in this cell population. It will be interesting to determine if Brm-Swi/Snf compensates for the loss of Brg1 in this context and how this coregulator influences Pdx1 action in adult islet  $\beta$  cells.

In contrast to the extensive interactions of Pdx1:Swi/Snf within the pancreas multipotent progenitor cell population, complex formation became restricted to the insulin<sup>+</sup> cells produced later in development and in islet  $\beta$  cells (Figures S3A and S3B). However, as islet somatostatin hormone<sup>+</sup>  $\delta$  cells and acinar cells produce low levels of Pdx1, these results indicate some change within Pdx1 (potentially a posttranslational modification), or simply the higher levels in  $\beta$  cells allows specific recruitment of widely distributed Brg1-Swi/Snf and Brm-Swi/Snf. Strikingly, knockdown of Brg1 and Brm in  $\beta$  cell lines revealed that Brg1 was important for coactivation of Pdx1 target genes (*Insulin*, *MafA*, and *Glut2*) and Brm corepression (*Insulin*, *Glut2*, *MafB*, and *Ucn3*) (Figures 5A–5D). The acute conditions of our analysis presumably enabled detection of this regulatory pattern despite an only 50% reduction in ATPase subunit levels, and not compensation by the unaffected coregulator that has been observed in vivo (Bultman et al., 2000; Smith-Roe and Bultman, 2013). Furthermore, Brg1 was found to directly bind within Pdx1-binding control-element-containing regions of these activated genes in ChIP assays (Figure 4; Bultman et al., 2000; Smith-Roe and Bultman, 2013; Willis et al., 2012).

Next, we examined if Pdx1 binding to Brg1-Swi/Snf and Brm-Swi/Snf was acutely regulated by changes in blood glucose levels in vivo, specifically examining low (fasting) to high (fed) conditions that influence Pdx1 activity in  $\beta$  cell lines. For example, *Insulin* gene transcription is enhanced by recruitment of the p300 coactivator, which catalyzes histone H4 hyperacetylation within the proximal promoter region that correlates with gene activation (Mosley et al., 2004). Conversely, Hdac1/2 recruitment by Pdx1 at non-stimulating, low-glucose levels inhibits transcription (Mosley and Ozcan, 2004). Similarly, Pdx1:Brg1 activator complex formation was rapidly and significantly increased in islet  $\beta$  cells by elevated blood glucose levels, while Pdx1:Brm repressor binding was unaffected (Figures 3A–3F). This was observed under circumstances where nuclear Pdx1, Brg1, and Brm levels were unaffected by changing glucose levels (Figure S7). We conclude that high-glucose concentrations amplified Pdx1:Brg1-Swi/Snf activator complex formation over Pdx1:Brm-Swi/Snf, causing transcriptional activation by directly influencing nucleosome occupancy and phasing in an ATP-dependent manner (Figures S6A and S6B). This represents a distinct mechanism from Pdx1-recruited p300 gene activation described above. Collectively, these



**Figure 5. The Pdx1-Recruited Brg1- and Brm-Containing Swi/Snf Complexes Appear to Play Opposing Regulatory Roles in  $\beta$  Cells**

(A–D) Treatment with (A) siBrg1 or (C) siBrm effectively reduced protein levels over scrambled RNAs in INS-1 cells, as demonstrated by immunoblot and densitometry analysis. (B) Expression of Pdx1-activated *Insulin*, *MafA*, and *Glut2* were significantly compromised following Brg1 knockdown, while (D) Brm knockdown had the opposite effect, causing upregulation of *Insulin*, *Glut2*, and non-Brg1 targets (*MafB* and *Ucn3*). The siRNA transfections were performed in triplicate on three separate occasions ( $n = 3$ ,  $*p < 0.05$ ). Error bars represent SD.

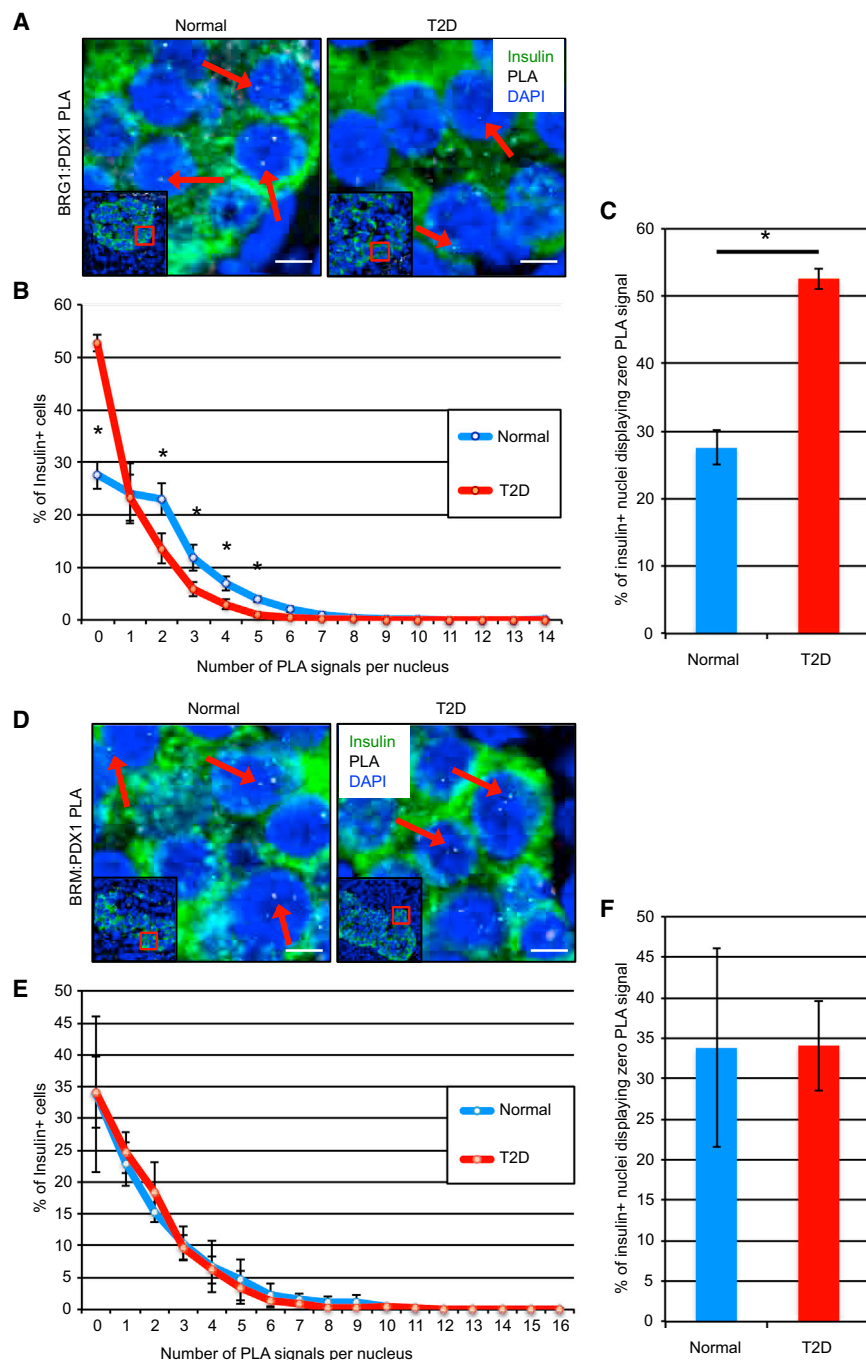
Peripheral insulin resistance and islet  $\beta$  cell dysfunction are the fundamental causes of T2DM, and symptoms often present slowly, after many years of progressive loss of  $\beta$  cell function and metabolic control. The initial response to peripheral insulin resistance is augmentation of Islet  $\beta$  cell mass and function (Talchai et al., 2009). However, the increasing metabolic demand imposed on  $\beta$  cells is strongly linked to their accumulation of destructive stress molecules, such as reactive oxygen species. The resulting oxidative stress conditions induce deleterious posttranslational modifications on proteins, affecting activity, stability, and subcellular localization. Indeed, a selective decline in transcription factors central to glucose sensing and insulin secretion is observed in T2DM islet  $\beta$  cells (i.e., MAFA, MAFB, PDX1, and NKX6.1) (Guo et al., 2013). Notably, MAFA and MAFB are highly susceptible to oxidative stress and their rapid loss is proposed to dictate first-phase insulin secretion defects early in disease progression. Persistent insults to PDX1 and NKX6.1, however, cause a more prolonged decline (years to decades) in activity, ultimately resulting in overt diabetes manifesting from severe  $\beta$  cell dysfunction.

Although it remains unclear how these T2DM-associated transcription factors are rendered inoperative in vivo, we speculate that pathophysiological conditions affect coregulator binding and, consequently, transcription factor function. Significantly, coregulator-catalyzed

results highlight how context-dependent recruitment of physiologically regulated and compositionally distinct Swi/Snf complexes dictate Pdx1 action and  $\beta$  cell function.

changes in epigenetic DNA methylation and histone modifications are found in T2DM islets, which influence promoter and enhancer structures driving islet-specific gene expression (Dayeh et al.,





**Figure 6. PDX1:BRG1-SWI/SNF Activator Levels Are Compromised in T2DM Islet  $\beta$  Cells**

(A–F) Representative PLA signals for PDX1 and (A) BRG1 or (D) BRM complexes in age-, sex-, and BMI-matched normal and T2DM human pancreas sections; arrows indicate PLA signals and lower magnification insets are provided to orient each image. Scale bars, 5  $\mu$ M. (B) PDX1:BRG1, but not (E) PDX1:BRM signal levels were significantly attenuated in T2DM  $\beta$  cell nuclei. (C) T2DM islets contain 2-fold more  $\beta$  cells displaying no detectable PDX1:BRG1 signal than normal controls. No significant difference in PDX1:BRM PLA signal (E) distribution per  $\beta$  cell or (F) number of  $\beta$  cells presenting zero was observed between T2DM and normal human islets. Paraffin sections from three individual T2DM and normal donors were independently analyzed and quantitated ( $n = 3$  for each donor of each type, \* $p < 0.05$ ). Error bars represent SD.

(i.e., Hdac1/2 and p300 recruitment [Mosley and Ozcan, 2004; Mosley et al., 2004]) and protein destruction (Pcif1-Cul3 [Clai-born et al., 2010]) are impacted by such events. We propose that PDX1:BRM-SWI/SNF activity contributes to the loss in T2DM  $\beta$  cell function (Figure S6). Moreover, conditions affecting PDX1 coactivator and corepressor recruitment occur relatively soon after exposure to insulin resistance in the context of T2DM disease process, and well before overt transcription factor loss.

Overall, our results clearly demonstrate the prominent role of Swi/Snf in regulating Pdx1 activity in  $\beta$  cells. Furthermore, we have uncovered that Pdx1 recruitment of Brg1-Swi/Snf and Brm-Swi/Snf is influenced by physiological and pathological settings, which are hypothesized to have significant implications on transcription factor activity and  $\beta$  cell function. In addition, this study illustrates how a ReCLIP/MS strategy can be used to identify coregulators of other islet-enriched transcription factors. Such knowledge will provide valuable mechanistic insight into

how these transcription factors regulate islet cell formation, function, and survival.

## EXPERIMENTAL PROCEDURES

### ReCLIP/MS

Mouse  $\beta$ TC-3 and human EndoC- $\beta$ H1 cells were grown as defined earlier [Nagamatsu and Steiner, 1992; Ravassard et al., 2011]. The ReCLIP procedure was performed as described [Smith et al., 2011] with minor modifications. Briefly, 14 15-cm culture plates of  $\beta$ TC-3 cells at 70% confluency ( $\sim 10^8$  cells)

2014; Parker et al., 2013). In addition, our PLA results illustrated that PDX1:BRG1 activator binding was diminished in T2DM  $\beta$  cells, while PDX1:BRM repressor complex formation was unaffected (Figures 6A–6F). Presumably, decreased PDX1:BRG1 activator formation simply reflects the diminished levels of PDX1 protein in T2DM  $\beta$  cells (Guo et al., 2013), while how BRM binding to PDX1 is selectively retained is unclear. Posttranslational modifications of PDX1 and/or SWI/SNF subunits likely influence their interactions, since, for example, Pdx1 transcriptional activity

were exposed to freshly made DSP (stock 20 mM in DMSO) diluted to a final concentration of 1 mM in PBS (pH 7.4) for 45 min at 37°C. Nuclear extract was prepared as previously described (Schreiber et al., 1989), except that DTT was withheld from the extraction buffer. The nuclear extract was incubated with either goat  $\alpha$ -Pdx1 (generated by Dr. Chris Wright, Vanderbilt University) antibody or goat IgG (control)-bound Protein G Dynabeads for 3 hr at 4°C, and then washed with RIPA buffer (100 mM NaCl, 1% Nonidet P40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris/HCl [pH 8.0], and 1 mM EDTA). Pdx1-binding partners were eluted with RIPA buffer supplemented with 200 mM DTT. Eluted proteins were analyzed via MudPIT (Multidimensional Protein Identification Technology) as previously described (Martinez et al., 2012) in the Vanderbilt University Medical Center Proteomics Core. MS analysis was performed on at least three independent ReCLIP preparations, with each showing similar enrichment of specific peptides.

### colIPs and Immunoblotting

$\beta$ TC-3 nuclear extracts were incubated with either goat  $\alpha$ -Pdx1 (generated by Dr. Chris Wright, Vanderbilt University), rabbit  $\alpha$ -Brg1 (Santa Cruz Biotechnology, H88-X), rabbit  $\alpha$ -Tif1 $\beta$  (Abcam, ab10483), rabbit  $\alpha$ -Mi2 $\beta$  (Abcam, ab72418) antibody, or species-matched IgG (control)-bound Protein G Dynabeads for 3 hr at 4°C, as described previously (Hunter et al., 2013). The immunoprecipitates were fractionated by SDS-PAGE (NuPAGE 10% Bis-Tris gels), transferred onto polyvinylidene fluoride (PVDF) membrane, and probed with the following antibodies: goat  $\alpha$ -Pdx1 (1:10,000); rabbit  $\alpha$ -Brg1 (Santa Cruz, H88-X, 1:5,000); rabbit  $\alpha$ -Brm (Cell Signaling Technology, 6889S, 1:2,000); rabbit  $\alpha$ -Tif1 $\beta$  (Abcam, ab10483, 1:2,000); rabbit  $\alpha$ -RBBP4 (Bethyl Laboratories, A301-206A, 1:2,000); rabbit  $\alpha$ -Mi2 $\beta$  (Abcam, ab72418, 1:2,000); rabbit  $\alpha$ -Beta-Actin (Cell Signaling Technology, 4967S, 1:2,000); HRP-conjugated  $\alpha$ -rabbit IgG (Promega, 1:2,000), and HRP-conjugated  $\alpha$ -goat IgG (Santa Cruz, 1:2,000). The experiments were performed at least three times and quantitated using NIH ImageJ software.

### Sucrose Gradient Ultracentrifugation and EMSA

Sucrose gradients were performed as previously described (Guo et al., 2010). Briefly,  $\beta$ TC-3 nuclear extract (1.5–2.5 mg) were separated over a 10%–35% sucrose gradient (4.5 ml total volume). Fractions (300  $\mu$ l each, excluding the first 500  $\mu$ l) were analyzed by immunoblotting, mouse *MafA* Region 3 element binding in EMSAs (Guo et al., 2010), and by immunoprecipitation.

### Immunofluorescence

Tissue fixation, embedding, and immunofluorescence labeling were performed as previously described (Matsuoka et al., 2003). The primary antibodies used were goat  $\alpha$ -Pdx1 (1:15,000), rabbit  $\alpha$ -Brg1 (1:300), rabbit  $\alpha$ -Brm (Abcam, ab72418, 1:300), and guinea pig  $\alpha$ -insulin (Dako, A0546, 1:500). The secondary Cy2-, Cy3-, or Cy5-conjugated donkey  $\alpha$ -rabbit,  $\alpha$ -guinea pig, and  $\alpha$ -goat IgGs were obtained from Jackson ImmunoResearch Laboratories. Nuclear counterstaining was performed using DAPI (Invitrogen). Immunofluorescence images were acquired by fluorescence microscopy using a Zeiss Axioimager M2 and processed by NIH ImageJ software.

### PLA

Paraffin-embedded human pancreas, mouse embryonic, and adult pancreas samples were analyzed by procedures described by the PLA kit manufacturer (Olink Bioscience), with minor modifications. Slides were de-waxed/rehydrated as previously described (Matsuoka et al., 2003). Heat antigen retrieval in 1  $\times$  TEG (25 mM Tris HCl (pH 8), 10 mM EDTA, and 50 mM glucose) was followed by three 10-min 1  $\times$  PBS washes. A 1% BSA/PBS blocking solution supplemented with 5% normal donkey serum was applied for 2 hr at room temperature. The following primary antibodies were incubated in a humidity chamber overnight at 4°C: goat  $\alpha$ -Pdx1 (1:15,000), rabbit  $\alpha$ -Brg1 (1:300), rabbit  $\alpha$ -Brm (Abcam, ab72418, 1:300), mouse  $\alpha$ -glucagon (Sigma-Aldrich, g2654-0.5ml, 1:4,000), and guinea pig  $\alpha$ -insulin (1:500). Immunofluorescence images were acquired on a Zeiss Axioimager M2 and processed for counting analysis using NIH ImageJ software. The discrete fluorescent nuclear PLA spots were counted from at least three pancreas sections per experimental animal or human donor. The Gift of Hope Organ Procurement Organization in Chicago generously provided human pancreata, which were obtained from three

normal and three T2DM de-identified cadaver donors: normal, two male and one female (59.3  $\pm$  8.5 years [range: 51 to 68], BMI 22.6  $\pm$  2.4 [range: 21.1 to 25.4]); T2DM, two male and one female (57.0  $\pm$  5.3 years [range: 51 to 61], BMI 25.9  $\pm$  7.1 [range: 21.2 to 34]).

### ChIP Assays

Mouse Min6  $\beta$  cells ( $\sim 4 \times 10^6$  cells) were 1% formaldehyde cross-linked, and the sonicated protein-DNA complexes isolated under conditions described previously (Gerrish et al., 2001). The fragmented chromatin was incubated with  $\alpha$ -Pdx1,  $\alpha$ -Brg1,  $\alpha$ -Brm, or species-matched IgG (Bethyl Laboratories), and the complexes isolated with BSA- or herring sperm single-stranded DNA (ssDNA) (Abcam, ab46666)-blocked protein-A Dynabeads (Invitrogen). Quantitative real-time PCR was performed using SYBR Green master mix in a Roche LightCycler 480II. The PCR primers were as follows: MafB –742 to –938 (site1), forward 5'-TTAGCGCAGACAGAGCTACCGAAA-3', reverse 5'-ATACTCTTTACACTCCACCCTCG-3'; Glucagon +70 to –148 (contains G1 element), forward 5'-CGTAAAAAGCAGATGAGCAAAGTG-3', reverse 5'-GAA CAGGTGTAGACAGAGGGAGTCC-3'; Pax4 –1,841 to –1,966 (contains  $\beta$  cell-specific enhancer), forward 5'-CCAACGATCCAGGCTCTACATC-3', reverse 5'-CGGGTTTGGGGCTAATTGTCC-3'; Pdx1 –2,471 to –2,598 (contains Area I), forward 5'-TGCTCGGGAAGGCTCTTG-3', reverse 5'-CCAT CAGGTGGCTAAATCCATTATG-3'; IAPP –97 to –190, forward 5'-TCACCCA CACAAAGGCACTCAG-3', reverse 5'-GGTTTCATTGGCAGATGGAGC-3'; MafA R3, forward 5'-CTGGAAGATCACCGCACA-3', reverse 5'-ATTACCA AGCCCCAAACG-3'; Insulin (proximal promoter), forward 5'-GCCATCTGCT GACCTACCC-3', reverse 5'-CCCCTGGACTTTGCTGTTT-3'; and Albumin –3,164 to –3,342 (distal TAAT-containing region), forward 5'-TGGGA AACTGGGAAAACCATC-3', reverse 5'-CACTCTCACACATACACTCTCTGC TG-3'. Experiments were performed with at least three independently isolated chromatin preparations.

### siRNA Knockdown and RNA Analysis

Rat INS-1 cells ( $7.5 \times 10^5$  per well) were seeded into six-well plates and targeting Brg1, Brm, or scrambled control siRNAs (Dharmacon) introduced using Lipofectamine 2000 (Invitrogen). RNA and immunoblot protein studies were performed 72 hr following treatment. Cellular RNA was isolated using the QIAGEN RNeasy Mini Kit. cDNA was prepared from RNA using the iScript cDNA synthesis kit (Bio-Rad), and quantitative real-time PCR was performed using SYBR Green master mix and a Roche LightCycler 480II. The experimental data were normalized to *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*) mRNA levels, and relative changes were calculated by the comparative DCt method (Chakrabarti et al., 2002).

### Glucose Treatment Conditions

C57BL/6 mice (Charles River Laboratories) were fasted from 18:00 to 10:00 hr, and then blood glucose measurements were taken from the tail tip using an Aviva glucometer (Accu Chek). Glucose was administered to the high group by intraperitoneal injection of a 20% glucose solution to achieve a final dose of 2 g/kg body weight. Glucose measurements were taken at 0, 15, and 30 min post-injection. Pancreata were harvested from each group and tissue embedded as previously described (Matsuoka et al., 2003).

### Statistics

Data are expressed as the means  $\pm$  SD. p values were calculated with a Student's two-tailed test. Results were considered significant at  $p < 0.05$ .

### Study Approval

The institutional review board at the University of Chicago and Vanderbilt University approved the use of human tissues in these studies. The Vanderbilt University Institutional Animal Care and Use Committee approved all studies involving animals.

### SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.02.054>.

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